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1. Your reference
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2. Patent Application Number
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if known)*

If the applicant is a corporate body, give the country/state of its incorporation
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State:

4. Title of the invention

Antibiotic Monitor

5. Name of agent
"Address for Service" in the United Kingdom to which all correspondence should be sent

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Patents ADP number 1826001

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications filed in the last 12 months.

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BERESFORD & Co

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Patent disclosure

Antibiotic monitor

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Introduction

Antibiotics are drugs which are used to control bacterial infections in humans and animals (in the following referred to as the patient"). They are often used when infection is to great for the body to deal with or the immunsystem is suppressed.

An antibiotic is a selective poison. It has been chosen so that it kills the bacteria preferentially, but not the cells of the patient's body. Each different type of antibiotic affects different bacteria in different ways. Most antibiotics act on the bacterial wall synthesis or protein synthesis.

Clinically important antibiotics include penicillin, cephalosporin, griseofulvin, bacitracin, polymyxin B, amphotericin B, erythromycin, neomycin, streptomycin, tetracycline, vancomycin, gentamicin, rifamycin, to name but a few. Others have been isolated and new antibiotic agents are being produced.

Other compounds with antibiotic activity have been isolated from micro-organisms over the years, but only a few are clinically useful. The reason is that only compounds with selective toxicity can be used clinically – the compounds must be highly effective against a micro-organism but have minimal toxicity in humans.

Vancomycin is an antibiotic, in particular as it is sometimes the only available antibiotic that is effective against microbes which have multiple resistance against other antibiotics. These bacteria account for a large portion of all hospital acquired infections and hence vancomycin is widely used to control these infections.

Vancomycin is an aminoglycoside antibiotic. Its structure is shown in Figure 1. Its main target is the D-alanyl-D-alanine terminal dipeptide of peptidoglycan precursors, used by bacteria for constructing their cell walls. This prevents the reaction used to link peptidoglycan precursors from taking place. Vancomycin binds with the substrate, not the enzyme.

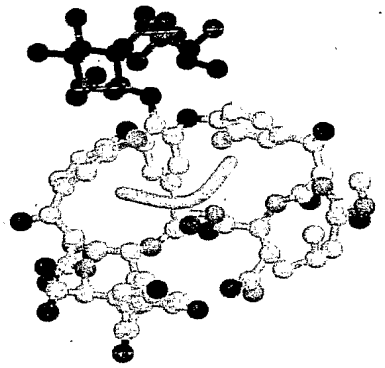

Figure 1: Ball and stick model of vancomycin. The backbone of the target molecule is shown in cyan.

Furthermore, the structure of vancomycin enables some modifications that allow it to retain its antibiotic properties, but overcome many of the resistance mechanisms observed to date. The molecule is a glyco peptide with the sites that bind to the peptidoglycan precursors located in the peptide core. Natural producers of the antibiotic add sugars to the hydroxyl group of one of the core amino acid side chains. Semi-synthetic vancomycin analogues can be produced by changing the nature of these sugar residues which are active *in-vivo* against many of the most important species of bacteria that are resistant to vancomycin.

To date only laboratory-based tests are available to monitor the level of these substances in the patient. These tests typically require a long turn around time. In order to achieve effective control of antibiotic drug delivery, monitoring methods and devices have to be developed which provide measurements close to the patient on a timescale compatible with the control of the delivery of the drug.

In the case of vancomycin, serum peak and trough concentrations are monitored routinely using laboratory tests. The bactericidal activity of vancomycin is concentration independent at serum concentrations greater than 5 μg/mL.The time above the minimum inhibitory concentration of the pathogen is the key to administering vancomycin effectively. Most susceptible organisms are killed when the trough concentration of vancomycin can be maintained between 5 and 15 μg/mL. Vancomycin therapy can affect renal, auditory, or central nervous system functions and can cause toxic adverse events or hypersensitivity reactions. Red-man symptome, nephrotoxicity, ototoxicity, thrombophlebitis and reversible neutropenia are examples of adverse effects observed during vancomycin treatment. Some of these are enhanced by the concurrent use of other medication. Hence, means to monitor the vancomycin concentration close in serum to real time are highly desirable.

In order to control and optimise the delivery of antibiotics, such as vancomycin, clinicians or more generally care staff require a monitoring device which will monitor the level of antibiotics, their derivatives, their target bacteria and/or their derivatives in biological fluids in near real time without any significant side effects for the patient. This is particularly important as many of these substances are toxic and may be harmful to the patient if the wrong dose is delivered.

The invention

The invention disclosed in this document is a monitoring device which will monitor in biological samples the level of antibiotics, their derivatives, their target bacteria, their derivatives and/or substances given off by the bacteria. Examples of biological samples include, but are not limited to blood, serum, plasma, interstitial fluids, saliva, cerebrospinal fluid, breath or other fluids, which may be optionally purified to remove, for example, red blood cells, platelets etc. The measurement approaches described are applicable to humans and animals. "). Methods of manufacturing or operating the device are also disclosed.

In one embodiment the device consists of a sensor element which is functionalised with a chemical recognition element which preferentially reacts with or binds one or more antibiotic(s) being used to treat the patient, for example, but are not limited to, vancomycin and the other antibiotics mentioned above, to one or more of their derivatives, to their target bacteria, their derivatives and/or substances given off by the bacteria. All these substances are generally referred to as the "analyte(s) of interest" in this document. The chemical recognition element may be a biologically derived substance, such as an enzyme, antibody, protein, micro-organism, cell, bacterium or virus to name but a few, or an artificial or synthetic receptor, such as a molecularly imprinted polymer (MIP). The latter are particularly attractive in the context of this invention as they have advantages with respect to biologically derived receptors in terms of robustness, cost and difficulties associated in raising a suitable biologically derived receptor.

One further example of a recepetor which can be used for vancomycin is the D-alanyl-D-alanine terminal dipeptide of the peptidoglycan precursor.

A further embodiment of the invention employs cells, bacteria or other micro-organisms which respond to the analyte(s) of interest, in particular antibiotic(s), their derivatives, target bacteria and/or substances given off by the target bacteria as chemical recognition elements. The sensor element(s) monitor the activity of these cells, (receptor) bacteria or other micro-organisms, e.g. the electrical activity, metabolism, reproduction, substances or signals given off by them etc., or any other indicator which may provide an indication on the level of the analyte(s) of interest in the sample. The cells, bacteria or other micro-organisms may be grafted directly onto the sensing element(s) or may be enclosed on top of or in the vicinity of the sensing element(s), e.g. using a membrane which is semi-permeable to the analyte(s) of interest.

In another embodiment, the chemical recognition element(s) respond(s) to the analyte(s) of interest by releasing a substance which is detected by the sensing element, either directly or indirectly. In a further example of the invention, the recogntion element may provide a form of chemical amplification, e.g. release two molecules of the substance for every molecule of the analyte(s) of interest. The substance released may also be used to provide other benefits, e.g. it may be a form of medication beneficial to the patient.

The sensor may also operate in a competitive mode. In this mode of operation, the chemical recognition element(s) which respond(s) to one or more other substances which are displaced from the recognition element by the analyte(s) of interest, but not directly to the analyte(s) of interest. In this case, the sensor signal will decrease in the presence of the analyte(s) of interest as the substance(s) are displaced from the recogntion element(s). In one particular embodiment, semi-permeable membranes and/or other confinement structures may be used to trap the substance(s) close to the sensing elements. In another embodiment, the substance(s) may react with the analyte(s) of interest.

The sensor can use a wide range of transduction or sensing principles to detect the interaction of the chemical recognition element with the analyte(s) of interest. Transduction principles include, but are not limited to, amperometric, conductimetric, potentiometric (in particular, ion-sensitive field effect transistor, ISFET, or chemically modified field effect transistor, CHEMFET, or more generally any device where the input is a chemical reaction or the presence of a particular chemical in close proximity to the field effect device), gravimetric, thermal, optical, resonant or surface-acoustic wave detection.

The sensor may be combined with a sampling device which will enable the sampling of the respective fluid from the patient being treated. Of particular interest are patient-connected sampling systems, for example, arterial or venous lines, which enable the sampling of blood from the patient. Blood may be withdrawn once, repeatedly or periodically over the sensor or into a container (e.g. connected to the sensor or for transport to the sensor) in order to enable the analysis. Alternatively, the sensor may be associated with a bypass system, e.g. a bypass used in cardiac surgery.

After the analysis the blood may be returned to the patient or discharged, e.g. to waste. Other sampling methods include syringes, intra-cranial drains (e.g. for the analysis of cerebrospinal fluid), microdialysis probes or microneedles (e.g. to access interstitial fluids and/or blood). Others are known to those skilled in the art.

The sensor may be configured to analyse samples from the patient repeatedly or periodically. Alternatively, it may be a configured to be used once, e.g. as a disposable or test strip. In another embodiment, the sensor is incorporated into a larger instrument, for example, an in-vitro analyser, configured to be used once or a number of times. The sensor may also be operated in a mode where it is flushed with a suitable solution or mixture after each use, for example, to clean the sensor, to remove substances contained in the sample from the sensor or to enable samples to be analysed repeatedly.

Furthermore, the sensor may be used continuously. In this case, the affinity of the chemical recognition element can be adjusted to facilitate the operation of the sensor in this mode.

The analysis can be conducted on-line, which is of particular advantage for the control of antibiotics delivery. Alternatively, other embodiments of the invention may also be employed for off-line analysis.

The chemical recognition elements may be associated with the sensing elements in a variety of forms. They can be thin layers, in particular mono- or multilayers, of receptors or recognition elements deposited on the sensing elements. Alternatively, they may be membranes which respond to the presence of the analyte(s) or react with the analyte(s) in a known manner. Alternatively, membranes can act as filters which allow the analyte(s) to pass, while restricting the passage of other substance interfering with the measurement. The recognition elements may also take the form of particles contained within or confined below a membrane. Other forms are known to those skilled in the art of preparing and using these receptor materials.

Furthermore, the chemical recognition elements may react with the analyte(s) of interest to release another substance or generate an event which is detected by the sensor element.

In some cases, further purification and concentration of the analyte(s) of interest can be achieved *in situ* by encapsulating or covering the sensing elements in one or more material(s), solid or liquid, into which the analyte(s) of interest preferentially partition(s) over the test medium it is in. One particular example, is an analyte which is in a polar test medium, but which partitions preferentially into a non-polar solvent. A membrane may be used to enclose the partitioning material, if required. The membrane may be semi-permeable to the analyte(s) of interest. An illustration of one particular embodiment of this invention using this approach is shown in Figure 2.

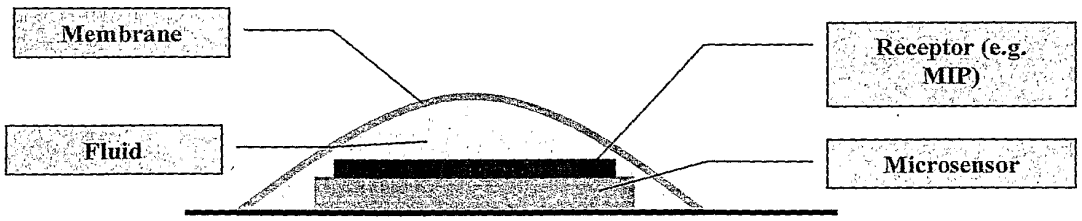

Figure 2: Schematic illustration of one particular embodiment of the invention.

One particular embodiment of the invention employs micromachined sensing elements to detect the analyte(s) of interest. Micromachined sensors are particularly attractive as they are of low cost and small size and, hence, can be used close to the patient, avoiding transport of the sample to be analysed from the patient to the analyser.

A further embodiment of our invention employs a silicon-based microsensor chip which incorporates one or more chemical sensing elements. A particular example of a multiple-analyte sensor is shown in Figure 3.

This particular chip employs potentiometric, in particular ISFETs and CHEMFETs, amperometric and conductimetric devices functionalised to respond to the analyte(s) of interest. However, the invention is not limited to multi-parametric micromachined chemical sensors and can employ a wide range of other microscopic and macroscopic sensors and transduction or sensing principles (see above).

The sensing elements may be functionalised to detect the analyte(s) of interest in a wide variety of ways, including, but not limited to deposition, evaporation, spin-coating, printing, ink-jet printing, dropping, spotting, centrifugation, screen printing, dripping, pipetting, droplet transfer (using e.g. a needle structure) etc. of suitable recognition elements or a mixture containing reagents which will lead to the creation of these elements.

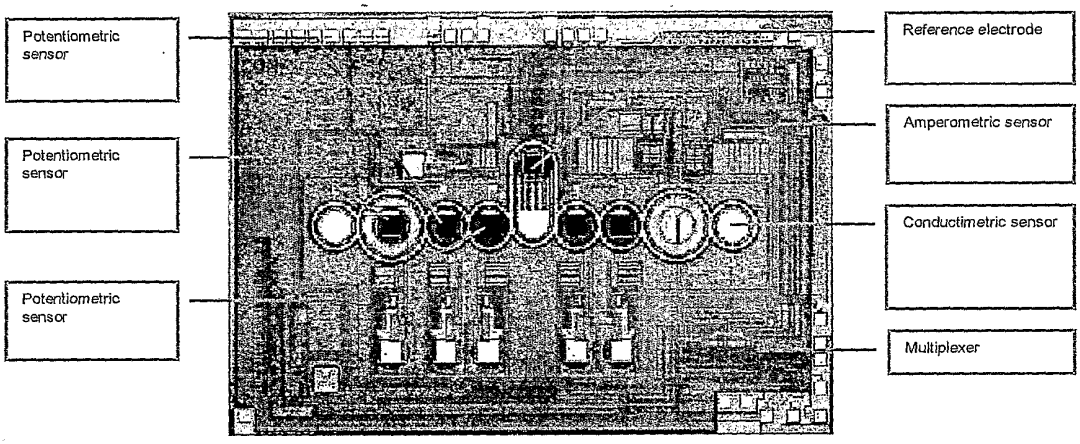

Figure 3: Example of a multi-parameter chemical sensor chip developed by Sphere Medical Ltd.

In addition to the approaches outlined above, a further method by which to functionalise the sensing element(s) on such a single- or multi-parametric chip is by the use of confinement structures on the device substrate around one or more features, e.g. sensor element(s). The structures may be circular or of any general shape which will be suited to the application in hand. They can be created during or following the manufacturing process of the device and can be made from a variety of materials, such as polymers, photoresists (e.g. polyimide or SU8), passivation materials (e.g. silicon oxide, nitride or oxynitride), metals, insulators or semiconductors. In general, the shape of the structures will be chosen to suit the size and shape of the transducers. These structures would act to contain the mixture of some or all of the reagents or components that will be used to create the chemical recognition element, e.g. the synthetic receptor(s), the partitioning material and/or membrane of the device or sensor. Due to the containment within the structure a larger number of functionalised sensor elements can be created in a given surface area on the substrate. Moreover, different mixtures can coexist on the surface of the substrate at the same time without mixing or cross-contamination. In addition, the confinement structures provide a means of achieving a uniform or complete coverage of the feature or sensor element even if parts of the mixture (e.g. a solvent) evaporate.

The confinement structure(s) may also provide means to improve the adhesion of the chemical recognition element on the substrate, sensor element or confinement structure, for example, by mechanical keying or the formation of chemical bonds.

Single and multiple dispensing heads may be used in order to enable serial or parallel deposition into the confinement structures. An example of such a deposition process is shown in Figure 4.

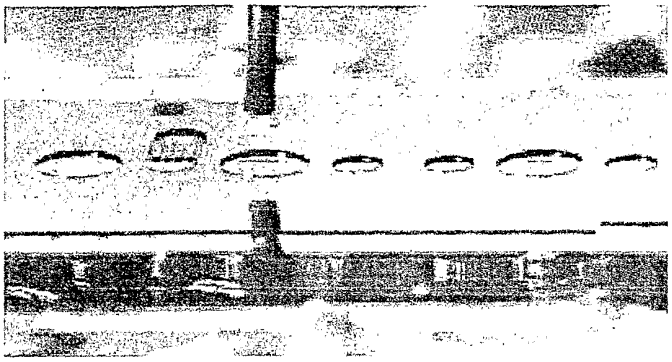

Figure 4: Deposition of materials into confinement structures around sensor elements on the chip shown in Figure 3 using a microspotter.

The confinement structures may also be used to aid the deposition of other material(s), for example electrolytes and the partitioning material(s), associated with the sensing element(s) for the analyte(s) of interest. An illustrative example of this embodiment is shown in Figure 5. Different confinement structures may contain different materials, e.g. partitioning materials. It is therefore possible to create different environments, e.g. polar and non-polar, on the same substrate.

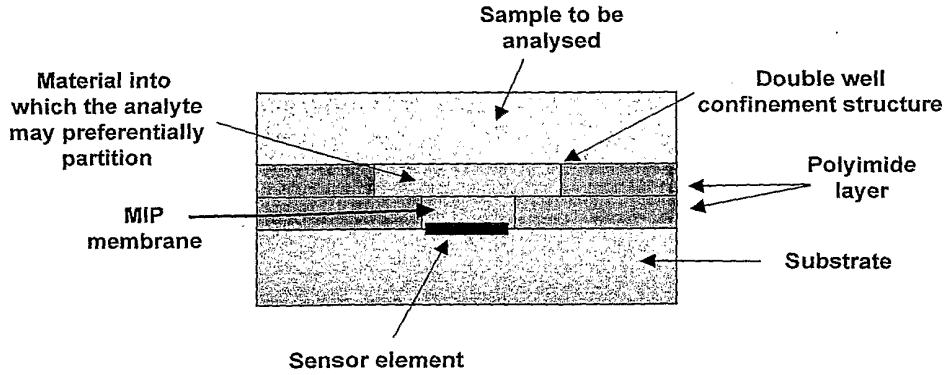

Figure 5: Schematic representation of an example of one embodiment of the invention where the sensing element is functionalised with a MIP and a material is deposited on top of a sensor element into which the analyte(s) of interest preferentially partitions.

This approach may be particularly advantageous for the detection of substances which exist as emulsions in a solvent.

In addition to providing preferential partitioning of analyte(s) into structures and materials associated with particular sensing elements, this approach may also provide a specific or desirable environment around a sensing element or receptor, e.g. to

improve its performance. For example, many MIPs have been designed or optimised for operation in non-polar media. By providing locally a non-polar environment on the substrate around the receptor, these MIPs may be employed in a chemical sensor operating in polar solvents for analyte(s) which will partition into the non-polar material.

In order to eliminate spurious effects associated, for example, with temperature fluctuations, it is generally advantageous to combine two identical transducer devices or sensing elements, only one of which is sensitive to the analyte(s) of interest, and to carry out differential measurements. The differences in the response of the two is therefore derived from the analyte(s) of interest; other interfering effects are fully or largely compensated for.

Using the present invention, an additional advantage may be obtained by carrying out a differential measurement on two transducers or sensing elements that are identical except for the fact that one is coated with a molecularly imprinted material and the other is coated with a material of identical composition, polymerised and/or crosslinked in the absence of the template molecule (see Figure 6 for a schematic illustration of one particular embodiment of this aspect of the invention). The reason for this is that a MIP material can have, besides the binding sites specifically suited to the analyte(s) to be detected, non-specific sites which can bind other molecules. On the other hand, the material polymerised in the absence of the template possesses only non-specific sites. It is thus possible to compensate either fully or partially for the interference which may be due to molecules other than the analyte(s), which become bound to the sensitive layer by non-specific interactions. In the present invention, the two transducers or sensing elements can be combined on the same substrate by creating confinement structures around the individual sensor elements and realising the template-imprinted MIP and the material crosslinked in the absence of a template in the confinement structures around the two respective sensing elements.

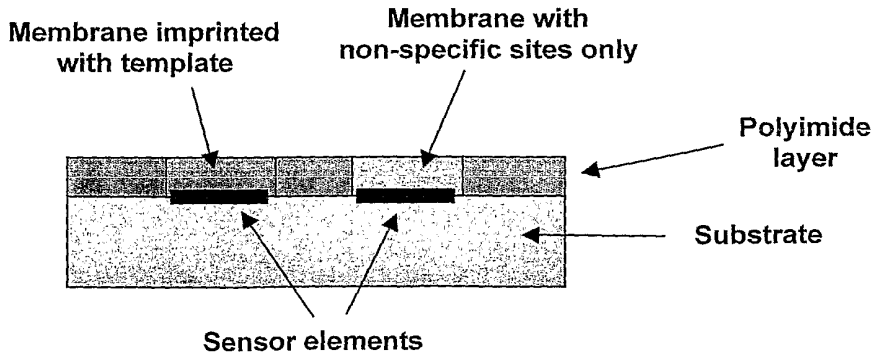

Figure 6: Example of a substrate with two identical sensing elements, one of which is functionalised using a MIP imprinted with the analyte(s) of interest, while the other is functionalised with a membrane of identical composition, but polymerised in absence of the template. This second sensing element serves as a reference sensor to identify and account for non-specific interactions of the analyte(s) and test medium with the MIP.

Rather than providing a reference transducer with a receptor material which is crosslinked in the absence of a template molecule, further embodiments of the invention employ one or more reference sensor(s) functionalised with a receptor material sensitive to any of the following species or any combination thereof:

- One or more interfering species to the analyte(s) of interest;
- One or more products of chemical reactions involving the analyte(s) of interest, e.g. a metabolite;
- Derivatives of the analyte(s) of interest;
- The target bacterium or bacteria, derivatives thereof and/or substances given up by them;
- Any other chemical species which may affect the sensor operation.

Other reference sensors may be created from sensing elements without partitioning material or with different partitioning material. Yet another approach involves the creation of a reference structure by the functionalisation of a sensing element with a MIP which is pre-loaded with the analyte(s) of interest or a derivative, i.e. only has non-specific site available for binding.

In the simplest embodiment, the signal from the reference sensor may be subtracted from that of the sensor element which is functionalised to the analyte(s) of interest. However, more elaborate compensation schemes may be employed, known to those skilled in the art.

The device may also contain means for temperature measurement and temperature control. For example to enable measurements to be carried out at a certain temperature or to change the temperature.

In addition to measuring only one or more antibiotic(s), or more generally, the analyte(s) of interest, the sensor may be configured to measure other or a wider range of substances, for example,

- Other drugs;
- Substances interfering with the operation of the antibiotic;
- Substances which cause, intensify or amplify side effects or cross-sensitivities when present together with the antibiotic(s),
- Bacteria, their derivatives or substances given off by them;
- Disease markers or blood parameters (such as dissolved gases, pH, electrolytes).

Multi-parameter measurements of this type may be accomplished using a micromachined sensor chip, for example, a chip as shown in Figure 3.

More generally, the invention also comprises measurement systems which monitor the level of analyte(s) of interest, e.g. the antibiotic(s), in a patient together with other

parameter which characterise the health of a patient, monitor particular markers indicating disease states or direct the patient's treatment.

By simultaneously measuring concentrations of the analyte(s) of interest in other tissues, fluids or body compartments it is possible to determine the kinetic profile of analyte(s) within the body. Potentially, an extremely useful approach would be to measure either separately or simultaneously related metabolites of the analyte(s) of interest to give information on the physiological passage/pharmacokinetics of the analyte(s).

Information derived from such a sensing system could be used to provide the input to an expert system to guide the anaesthetists or enable closed-loop drug administration when coupled with the appropriate administration device and control algorithm.

Another embodiment is shown schematically in Figure 7. It consists of a measurement system which provides on-line and on-demand measurement of the level of the analyte(s) of interest, e.g. antibiotic(s), and/or other parameters in the patient's blood. It comprises a disposable sensor, for example, a packaged analysis chip, as shown in Figure 3, functionalised for the detection of the analyte(s) of interest and other desired parameters, which is integrated into or connected to a vascular access device, e.g. a cannula. Depending on the application the sensor may be connected to an artery, vein or other sampling site of the patient. Alternatively, it may be integrated or connected to an existing monitoring system attached to the patient, for example, but not limited to, an invasive blood pressure monitoring system, as shown in Figure 7. The sensor may be connected to an electronics unit which will analyse the signals and compute the desired output. The output may be displayed on a local display associated with the electronics. The electronics unit may also be connected to the bedside vital-signs monitor in order to provide connectivity to the hospital information system for display, trending, data storage, data analysis and access to the electronic patient records.

The electronics or parts thereof may be integrated into the sensor package or form a separate unit.

Whenever required, blood is sampled from the patient and flushed over the sensor. Once the sample is analysed, the blood may be flushed back into the patient or to waste. After the sample is analysed, a suitable solution may be flushed across the sensor, e.g. to clean the sensor, to remove substances contained in the sample from the sensor or to enable the sensor to be used repeatedly.

Sampling can occur manually, for example using a syringe connected to the sampling port shown in Figure 7 to withdraw blood from the patient, or automatically, using, for example, a pump or syringe pump connected to the line. The benefits of this system over existing solutions are: improved patient care, reduced blood contact and infection risk to patient and caregiver, reduced blood withdrawal from the patient, and reduced cost.

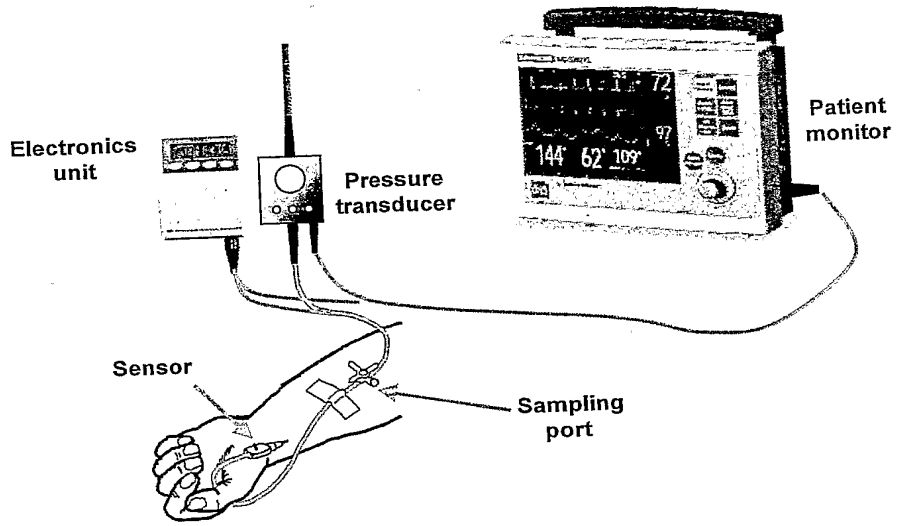

Figure 7: Illustration of a monitoring system attached to a vascular access line.

The sensor can be a disposable device which is used once, repeatedly or periodically. Of particular advantage is the use of a per-patient disposable sensor which is used repeatedly over a period of time, for example, minutes, hours, days, weeks or longer, or the while the patient is sedated or within a hospital or care environment. The menu of sensors on the chip will be configured for specific applications.

Also, MIP materials represent only one class of recognition element which can be used in conjunction with the invention. In this document, MIP is used for the purpose of illustration. Other materials, in particular other biologically derived or synthetic receptors, may be employed instead of or in addition to MIP.

In addition to antibiotics the invention disclosed in this document can also be used to monitor the level or concentration of so called predatory bacteria or other substances or organisms which can be harnessed to tackle infections in place of antibiotics. Bacteria, such as the *Bdellovibrio* bacterium, can be useful to fight other bacteria and infections instead of standard chemical antibiotics. The *Bdellovibrio* bacterium senses the presence of other bacteria, invades, and destroys them.